

Executive Summary

In response to the worsening water quality of the marine shellfish growing waters of Henderson Inlet, Thurston County Environmental Health Division conducted this study to identify the sources of pollution that impact Henderson Inlet waters. This study was designed to find the difference between human and animal contributors, evaluate the impact of each source, and locate the point of impact of each of the sources. Water (marine and freshwater), marine sediment, and shellfish tissue were sampled at different locations within the watershed.

The method used to identify the sources of pollution is called Microbial Source Tracking (MST). The MST is based on the use of genetic fingerprinting of *Escherichia coli* (*E. coli*) bacteria strains isolated from water and source samples by a method called ribotyping. It is the DNA fingerprint of the *E. coli* that lives within the animal that is typed, not the DNA of the specific animal. Some of the *E. coli* bacteria found in different warm-blooded animal groups, including humans, have genetic differences. It is thought that these distinctions in bacteria occur because the intestinal environments differ between animal groups. It is in identifying these genetic differences that it is possible to associate bacteria with sources of fecal pollution.

In order to be successful with this method, it is necessary to build a library of fingerprints from known sources. It is these fingerprints from the database library of known sources that are matched with the environmental samples collected. The larger and more diverse the library, the more likely a match can be made. The library used for this study currently has approximately 65,000 fingerprints called source isolates. An additional 100 source isolates were added from local collection of fecal samples from a variety of domestic animals, wildlife, and human sewage.

The work to accomplish this project was a joint effort between Thurston County Environmental Health staff, Dr. Mansour Samadpour, the University of Washington, and volunteers, who collected the fecal samples in order to build this local library of *E. coli* DNA patterns. The County collected and cultured all of the samples, the environmental samples. Dr. Samadpour and the University of Washington cultured the fecal samples, and typed and matched the *E. coli*.

The study area was southern Henderson Inlet near Washington State Department of Health's marine monitoring station #5. This station's commercial shellfish status was downgraded to *prohibited* in October 2000. DNA typing was used to test for bacterial pollutants affecting the water, sediment, and shellfish tissue of marine station #5. Water from the 3 creeks most likely to influence station #5 was also tested.

A bacterial pathogen study was done on a set of environmental samples from the study area. Because fecal coliform and *E. coli* are used as indicator organisms, it is of interest to know if there are specific human bacterial pathogens present in the samples collected.

Sampling for this study began in February 2000 and was completed in May 2001. Four sampling events occurred in 2000 with the remainder being completed during wet weather in 2001. Sampling was done during saturated soil conditions when run-off of nonpoint pollution is most likely to occur. The study was designed to obtain 100 isolates each for sediment, shellfish tissue, Woodland creek, and small creeks entering the cove at Station #5. Two hundred (200) isolates were to be typed for the marine water. With the exception of the shellfish tissue, which had 89 isolates, all exceeded the designed number of isolates. A total of 943 isolates were typed. Matches were made for 85.7% of the isolates; only 14.3% of the isolates could not be matched to any fingerprint within the library. Woodland Creek had the highest percent of matches – 91.7%; the marine water had the highest percent of unmatched – 18.0%.

Overall, a total of 27 source types of fecal pollution were identified. They were avian, beaver, bovine, canine, cat, deer, dog, duck, duck-goose, feline, goose, horse, human, marine mammal, multiple species, muskrat, opossum, otter, porcupine, poultry, rabbit, raccoon, rodent, seagull, sea lion, seal, and unknown.

The raw data has been analyzed and is presented in a variety of formats:

- Total number of isolates
- Number of source types
- Frequency of bacterial source type occurrences
- Comparison of sampling during *conditional closure* conditions (wet weather) and *open* conditions (dry weather)
- Comparison of sampling under *ebb* vs *flood* tide sampling conditions.

A significant aspect of the study was the percent of matches that were made – 86%. The project proposal had estimated between 40 and 60%. The high number of matches is due both to the size of the library, which is around 65,000 isolate patterns, and to the rigor of the study design.

The primary observation of the data regardless of sampling site – this is nonpoint pollution. There is a bit of everything. However, in analyzing the three water bodies sampled, it can be determined that the Swayne creeks are of a rural nature and Woodland Creek is of an urban nature. The source types found in Swayne creek samples represent animals found in rural areas – birds, deer, canines, and rodents. The types in the Woodland Creek samples reflected the urbanization of the watershed with people and dogs being predominant source types.

This is a study of Henderson Inlet only. Though much can be learned from this body of work, it is a picture of Henderson Inlet. The specific results of this work can not, and must not, be transferred to another watershed.

This is a qualitative study. It was designed to differentiate between human and animal sources and evaluate the frequency of those sources. Each set of site-specific results was evaluated for its impact on, and relationship to, the marine water quality and shellfish beds. The study was not designed to quantify the fecal loading of the sources. This is important to understand when reading the full report. One animal source may occur more frequently, but individually contribute less fecal matter. For example, one bird has much less fecal load contribution than one human. A study designed to calculate fecal loading contributions of individual sources would be more extensive and expensive.

The results of this study can help in development of continued remedial activity to improve water quality within Henderson Inlet. Of the predominant animal sources, birds are always present and everywhere – on land, on water, and in the air dropping their fecal matter at all sites. As a source, they are relatively beyond control other than to discourage grazing of migratory birds. Unknowns, until identified, are unmanageable. To identify these and reduce the number of unmatched isolates, more known *E. coli* fecal sources need to be typed and added to the library.

Human sources are the next most prevalent source. Human bacteria enters surface water, and subsequently sediment and tissue, through fecal contamination from failing septic systems, failing sewer lines, and direct deposition from humans and diapers. There are previously developed and proven programs that can be implemented that can find and correct many of these human sources.

This particular microbial source tracking (DNA ribotyping) method is considered by some professionals to be an experimental methodology. Studies and methods have not been subjected to rigorous peer review, and statistical evaluation has not been applied to the uncertainties and limitations of the method. Until this occurs, some environmental health professionals are reluctant to accept the results and conclusions of any study in which this methodology is used. To local environmental health professionals this methodology promises to be a valuable tool to use in order to prioritize remedial and preventative water quality work.

Specific conclusions from the study are --

- The percentage of matches with known sources was high – 86%.
- There must be an on-going effort to collect and type *E. coli* of fecal samples from known sources in order to increase the size of the library.
- Marine water samples had the greatest percentage of isolates without a match - 18%.
- This study represents only the Henderson Inlet study area.
- The study results are a 'picture' of nonpoint pollution. Twenty-seven (27) source types were identified.
- The results for the Swayne creeks were characteristic of its rural watershed.
- The results of Woodland creek were characteristic of its urban watershed.

- The results of the marine water were reflective of its complexity.
- Avian, human, canine, and unknown source types were found during more than half the water sampling events. These were the most frequently found source types.
- Overall, human, beaver, and bovine isolates were always more prevalent during wet sampling conditions. Feline, goose, and multiple species types were more prevalent during dry sampling conditions.
- Bovine source types were found in the marine water only during wet sampling events.
- On a flood tide and when the inlet is closed to commercial shellfish harvest, the marine water human source types were found more frequently than any other type at the marine station.
- Marine mammal source types were found more often during dry weather sampling under ebb tide conditions.
- Sediment samples had the least fingerprint 'uniqueness' of the sampling sites. There were more duplicate fingerprints within a given grab sample.
- The oyster sampling events produced such low number of isolates that no conclusions about the results can be made.
- The bacterial pathogen scan produced limited data. Human pathogens were found.
- This method shows promise as an effective tool for the complex task of identifying the sources of nonpoint pollution.
- The results of such studies can help prioritize local remedial efforts.

As a result of this study, the following recommendations are presented:

- State and local agency support is needed to fully develop the DNA ribotyping method for source identification of nonpoint pollution. Development of standard methods is needed in order that data can be shared, reviewed, and have professional support.
- State and local agencies in concert with researchers should develop a feces collection program in order to expand the DNA fingerprint library database.
- Due to the definite presence of human source types, the county and city should adopt a risk-based human waste assessment program that would include both evaluation of septic systems, as well as evaluation of the municipal sewer systems.
- Thurston Conservation District should continue development and management of conservation plans for watershed farmers and livestock owners so that best management practices that protect water quality are implemented and maintained.
- Area stormwater managers should discuss the possibility of conducting typing studies to categorize fecal sources within stormwater discharges.
- Advocate proper disposal of dog waste through state and local public education programs.
- Due to the limited data from sediment and oyster samples, future study designs should be modified to either omit these types of samples, increase the number of specimens collected, and/or add sampling events.
- Due to the limited data from the bacteriological pathogen scan, future studies having similar objectives should consider the value of the scan.

Terminology

Clonal type – a strain of bacteria that has so many identical characteristics (including their DNA fingerprints) that they have most likely originated from a common ancestral cell.

DNA – deoxyribonucleic acid; DNA is the chemical name for the genetic elements that all living cells have (their chromosomes).

DNA Fingerprint Library – a database of genetic fingerprints, in this instance an *E. coli* DNA Fingerprint Library.

Environmental samples – samples collected in the field as part of the study.

Escherichia coli – a fecal bacteria; commonly carried by humans and animals, most *E. coli* strains are not harmful to humans, a few, like *E. coli* O157:H7 are human pathogens. *E. coli* is used as an indicator organism for assessing the microbiological quality of water.

Isolate – a pure culture of bacteria that has been established in a laboratory.

Microbial Source Tracking (MST) – a methodology used to identify the sources of microbial pollution and to quantify their impact.

Transient clones – clonal types that are present in more than one different group or species of warm-blooded animals; referred to as 'transient' in the data tables.

Resident clones- clonal types that are shared by members of one source type; referred to as 'resident' in the data tables.

Prevalence – how often something is occurring.

Ribotyping – the specific methodology of genetic fingerprinting of bacteria.

Source types – the various types and groupings of warm-blooded animals that are sources of fecal nonpoint pollution.